

FILE 'REGISTRY' ENTERED AT 09:10:52 ON 10 MAR 2004

=> S 1.1.5.2

L1 0 1.1.5.2

=> S 1.1.5.2/CN

L2 0 1.1.5.2/CN

=> S PPQ-DEPENDENT (3A) GLUCOSE

6 PPQ

14228 DEPENDENT

0 PPQ-DEPENDENT

(PPQ (W) DEPENDENT)

24375 GLUCOSE

L3 0 PPQ-DEPENDENT (3A) GLUCOSE

=> S PYRROLOQUINOLINE

L4 265 PYRROLOQUINOLINE

=> S PYRROLOQUINOLINE AND (GLUCOSE DEHYDROGENASE)

265 PYRROLOQUINOLINE

24375 GLUCOSE

37441 DEHYDROGENASE

96 DEHYDROGENASES

37441 DEHYDROGENASE

(DEHYDROGENASE OR DEHYDROGENASES)

176 GLUCOSE DEHYDROGENASE

(GLUCOSE (W) DEHYDROGENASE)

L5 14 PYRROLOQUINOLINE AND (GLUCOSE DEHYDROGENASE)

=> S GLUCOSE DEHYDROGENASE/CN

L6 5 GLUCOSE DEHYDROGENASE/CN

=> D 1-5

L6 ANSWER 1 OF 5 REGISTRY COPYRIGHT 2004 ACS on STN

RN 81669-60-5 REGISTRY

CN Dehydrogenase, glucose (pyrroloquinoline-quinone) (9CI) (CA INDEX NAME)

OTHER NAMES:

CN β -D-Glucose dehydrogenase

CN D-Glucose dehydrogenase

CN E.C. 1.1.99.17

CN GlucDor

CN **Glucose dehydrogenase**

CN Glucose dehydrogenase (PQQ dependent)

CN Glucose dehydrogenase (pyrroloquinoline quinone)

CN PQQ glucose dehydrogenase

CN PQQ-dependent glucose dehydrogenase

CN Pyrroloquinoline quinone glucose dehydrogenase

CN Pyrroloquinoline quinone-dependent glucose dehydrogenase

CN Quinoprotein D-glucose dehydrogenase

CN Quinoprotein glucose dehydrogenase

MF Unspecified

CI MAN

LC STN Files: AGRICOLA, BIOBUSINESS, BIOSIS, CA, CAPLUS, CEN, CIN, PIRA,
PROMT, TOXCENTER, USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

216 REFERENCES IN FILE CA (1907 TO DATE)

11 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA

216 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L6 ANSWER 2 OF 5 REGISTRY COPYRIGHT 2004 ACS on STN
 RN 37250-84-3 REGISTRY
 CN Dehydrogenase, glucose (acceptor) (9CI) (CA INDEX NAME)
 OTHER NAMES:
 CN β -D-Glucose dehydrogenase
 CN D-Glucose dehydrogenase
 CN Dehydrogenase, glucose (Aspergillus)
 CN E.C. 1.1.99.10
 CN **Glucose dehydrogenase**
 CN Glucose dehydrogenase (decarboxylating)
 CN Glucose:methylviologen oxidoreductase
 MF Unspecified
 CI MAN
 LC STN Files: AGRICOLA, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CAPLUS, CEN,
 CIN, EMBASE, PIRA, PROMT, TOXCENTER, USPAT2, USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

44 REFERENCES IN FILE CA (1907 TO DATE)

44 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L6 ANSWER 3 OF 5 REGISTRY COPYRIGHT 2004 ACS on STN
 RN 37250-50-3 REGISTRY
 CN Dehydrogenase, glucose (nicotinamide adenine dinucleotide phosphate) (9CI)
 (CA INDEX NAME)
 OTHER NAMES:
 CN β -D-Glucose dehydrogenase
 CN D-Glucose dehydrogenase
 CN E.C. 1.1.1.119
 CN **Glucose dehydrogenase**
 CN Glucose dehydrogenase (nicotinamide adenine dinucleotide phosphate)
 CN NADP-dependent glucose dehydrogenase
 MF Unspecified
 CI MAN
 LC STN Files: AGRICOLA, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CAPLUS, CEN,
 CHEMCATS, CIN, EMBASE, MSDS-OHS, PIRA, PROMT, TOXCENTER, USPAT2,
 USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

70 REFERENCES IN FILE CA (1907 TO DATE)

2 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA

70 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L6 ANSWER 4 OF 5 REGISTRY COPYRIGHT 2004 ACS on STN
 RN 37250-49-0 REGISTRY
 CN Dehydrogenase, glucose (nicotinamide adenine dinucleotide) (9CI) (CA
 INDEX NAME)
 OTHER NAMES:
 CN β -D-Glucose dehydrogenase
 CN D-Glucose dehydrogenase
 CN E.C. 1.1.1.118
 CN **Glucose dehydrogenase**
 MF Unspecified
 CI MAN
 LC STN Files: AGRICOLA, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CAPLUS,
 CASREACT, CEN, CIN, EMBASE, PIRA, PROMT, TOXCENTER, USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

100 REFERENCES IN FILE CA (1907 TO DATE)
6 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA
100 REFERENCES IN FILE CAPLUS (1907 TO DATE)

L6 ANSWER 5 OF 5 REGISTRY COPYRIGHT 2004 ACS on STN
RN 9028-53-9 REGISTRY

CN Dehydrogenase, glucose (9CI) (CA INDEX NAME)

OTHER NAMES:

CN β -D-Glucose dehydrogenase

CN D-Glucose dehydrogenase

CN D-Glucose dehydrogenase (NAD(P))

CN E.C. 1.1.1.47

CN Glucose 1-dehydrogenase

CN **Glucose dehydrogenase**

CN Hexose phosphate dehydrogenase

CN NAD(P)-dependent glucose dehydrogenase

MF Unspecified

CI MAN

LC STN Files: AGRICOLA, BIOBUSINESS, BIOSIS, BIOTECHNO, CA, CAPLUS,
CASREACT, CEN, CHEMCATS, CHEMINFORMRX, CHEMLIST, CIN, CSCHM, EMBASE,
IFICDB, IFIPAT, IFIUDB, PIRA, PROMT, TOXCENTER, USPAT2, USPATFULL

Other Sources: EINECS**, TSCA**

(**Enter CHEMLIST File for up-to-date regulatory information)

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

871 REFERENCES IN FILE CA (1907 TO DATE)

29 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA

872 REFERENCES IN FILE CAPLUS (1907 TO DATE)

=> S 1.1.99.17

L7 1 1.1.99.17

=> D

L7 ANSWER 1 OF 1 REGISTRY COPYRIGHT 2004 ACS on STN

RN 81669-60-5 REGISTRY

CN Dehydrogenase, glucose (pyrroloquinoline-quinone) (9CI) (CA INDEX NAME)

OTHER NAMES:

CN β -D-Glucose dehydrogenase

CN D-Glucose dehydrogenase

CN **E.C. 1.1.99.17**

CN GlucDor

CN Glucose dehydrogenase

CN Glucose dehydrogenase (PQQ dependent)

CN Glucose dehydrogenase (pyrroloquinoline quinone)

CN PQQ glucose dehydrogenase

CN PQQ-dependent glucose dehydrogenase

CN Pyrroloquinoline quinone glucose dehydrogenase

CN Pyrroloquinoline quinone-dependent glucose dehydrogenase

CN Quinoprotein D-glucose dehydrogenase

CN Quinoprotein glucose dehydrogenase

MF Unspecified

CI MAN

LC STN Files: AGRICOLA, BIOBUSINESS, BIOSIS, CA, CAPLUS, CEN, CIN, PIRA,
PROMT, TOXCENTER, USPATFULL

*** STRUCTURE DIAGRAM IS NOT AVAILABLE ***

216 REFERENCES IN FILE CA (1907 TO DATE)

11 REFERENCES TO NON-SPECIFIC DERIVATIVES IN FILE CA

216 REFERENCES IN FILE CAPLUS (1907 TO DATE)

FILE 'CAPLUS' ENTERED AT 09:14:05 ON 10 MAR 2004

=> S L7

L8 216 L7

=> S PYRROLOQUINOLINE(3A) (GLUCOSE DEHYDROGENASE)

1206 PYRROLOQUINOLINE

154 PYRROLOQUINOLINES

1248 PYRROLOQUINOLINE

(PYRROLOQUINOLINE OR PYRROLOQUINOLINES)

363726 GLUCOSE

736 GLUCOSES

363868 GLUCOSE

(GLUCOSE OR GLUCOSES)

147520 DEHYDROGENASE

24090 DEHYDROGENASES

150540 DEHYDROGENASE

(DEHYDROGENASE OR DEHYDROGENASES)

2414 GLUCOSE DEHYDROGENASE

(GLUCOSE(W) DEHYDROGENASE)

L9 106 PYRROLOQUINOLINE(3A) (GLUCOSE DEHYDROGENASE)

=> S L8,L9

L10 241 (L8 OR L9)

=> S GLUCOSE

363726 GLUCOSE

736 GLUCOSES

L11 363868 GLUCOSE

(GLUCOSE OR GLUCOSES)

=> S MALTOSE;S ACINETOBACTER;S CALCOACETICUS

24426 MALTOSE

39 MALTOSES

L12 24432 MALTOSE

(MALTOSE OR MALTOSES)

5155 ACINETOBACTER

32 ACINETOBACTERS

L13 5159 ACINETOBACTER

(ACINETOBACTER OR ACINETOBACTERS)

L14 1796 CALCOACETICUS

=> S L10 AND L11

L15 234 L10 AND L11

=> S L15 AND L12

L16 18 L15 AND L12

=> S L16 AND L13

L17 11 L16 AND L13

=> S L16 NOT L17

L18 7 L16 NOT L17

=> D L17 1-11 CBIB ABS;D L18 1-7 CBIB ABS

L17 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

2004:41629 Document No. 140:107505 Water-soluble **Acinetobacter**

pyrroloquinoline quinone **glucose dehydrogenase**

mutants with improved **glucose** affinity for use in

glucose assay and sensor. Sode, Koji (Japan). PCT Int. Appl. WO

2004005499 A1 20040115, 39 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (Japanese). CODEN: PIXXD2. APPLICATION: WO 2003-JP8418 20030702. PRIORITY: JP 2002-196177 20020704; JP 2003-71760 20030317.

AB Water-soluble **Acinetobacter calcoaceticus pyrroloquinoline** quinone **glucose dehydrogenase** (PQQGDH-B) mutants with improved affinity for **glucose**, and encoding sequences are disclosed. Recombinant expression of those PQQGDH mutants, **glucose** assay kit and **glucose** sensor containing them are claimed. Based on the characterization of a PCR mutation of water-soluble **glucose dehydrogenase** possessing **pyrroloquinoline** quinone (PQQ), PQQGDH-B, the authors have constructed a series of variants with substitution of 186 to 206 residues. Glu192, Leu193, Asp167, or Asp452 were substituted with other amino acid residues. Those mutants showed much higher affinity for **glucose** and lower affinity for **maltose** or lactose compared to the wildtype. A double mutant Asp-167Glu/Thr366Asn showed a higher substrate specificity for **glucose** as well as activity. A **glucose** sensor consisting of mutant PQQGDH immobilized on an electrode via carbon paste was constructed.

L17 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

2003:947745 Document No. 140:14394 Modified **pyrroloquinoline**

quinone-dependent **glucose dehydrogenase** with superior

substrate specificity and stability. Takeshima, Seiji; Sogabe, Atsushi;

Oka, Masanori (Toyo Boseki Kabushiki Kaisha, Japan). Eur. Pat. Appl. EP

1367120 A2 20031203, 45 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK,

ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK,

CY, AL, TR, BG, CZ, EE, HU, SK. (English). CODEN: EPXXDW. APPLICATION:

EP 2003-11930 20030527. PRIORITY: JP 2002-152911 20020527; JP 2002-152913

20020527; JP 2003-80244 20030324; JP 2003-80310 20030324.

AB The present invention relates to modified **pyrroloquinoline** quinone-dependent **glucose dehydrogenase** (PQQGDH, from **Acinetobacter baumannii**) having lower activity with respect to disaccharides and/or greater stability than wild-type PQQGDH. Site-directed mutagenesis is used to generate mutants with improved substrate specificity based on amino acid substitutions at one or more of positions 67, 68, 69, 76, 89, 167, 168, 169, 341, 342, 343, 351, 49, 174, 188, 189, 207, 315, 245, 300, 349, 129, 130 and 131, and having an amino acid inserted between positions 428 and 429 of the mature enzyme. Improved thermal stability is achieved by amino acid substitutions at one or more of positions 20, 76, 89, 168, 169, 246, and 300. Expression vectors are designed for replication and enzyme production in *Pseudomonas putida*. The invention also provides assay kits and sensors for the detection of **glucose** using modified PQQGDH.

L17 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

2003:435231 Document No. 139:32504 Soluble **pyrroloquinoline** quinone-dependent **glucose dehydrogenase** mutants with improved specificity for **glucose** for use in **glucose** determination. Kratzch, Peter; Schmuck, Rainer; Bunk, Daniela; Shao, Zhixin; Thym, Detlef; Knappe, Wolfgang-Reinhold (Germany). U.S. Pat. Appl. Publ. US 2003104595 A1 20030605, 29 pp., Cont.-in-part of U.S. Ser. No. 710,197. (English). CODEN: USXXCO. APPLICATION: US 2001-82627 20011029. PRIORITY: EP 2000-123512 20001027; US 2000-710197 20001109; EP 2000-127294 20001219.

AB The present invention relates to improved variants of soluble **pyrroloquinoline** quinone (PQQ)-dependent **glucose dehydrogenases** (s-GDH), to genes encoding mutated s-GDH, to mutant proteins of s-GDH with improved substrate specificity for **glucose**, and to different applications of these s-GDH variants, particularly for determining concns. of sugar, especially of **glucose** in a sample.

L17 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

2002:716492 Document No. 137:243935 Disulfide bonded water-soluble **Acinetobacter pyrroloquinoline** quinone **glucose dehydrogenase** mutants with improved thermal stability for use in **glucose** assay and sensor. Sode, Koji; Igarashi, Satoshi (Japan). PCT Int. Appl. WO 2002072839 A1 20020919, 36 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (Japanese). CODEN: PIXXD2. APPLICATION: WO 2002-JP2124 20020307. PRIORITY: JP 2001-70413 20010313.

AB Water-soluble **Acinetobacter pyrroloquinoline** quinone **glucose dehydrogenase** (PQQGDH) mutants having disulfide bond-linked subunits with improved thermal stability, and their genes are disclosed. Recombinant expression of those PQQGDH mutants, **glucose** assay kit and **glucose** sensor containing them are claimed. Ser415Cys showed higher thermal stability than the wild type enzyme, retaining 90% of its activity at 70°C compared to 50% reduction at 60°C for the wild type. It also retained the similar level of catalytic activity. Asn340Cys/Tyr418Cys mutant also showed higher thermal stability compared to the wild type. They had similar substrate specificity to the wild type enzyme, showing activity toward **glucose**, **allose**, **3-o-methyl-D-glucose**, **galactose**, **lactose**, and **maltose**. A **glucose** sensor consisting of Ser415Cys PQQGDH immobilized on an electrode via carbon paste was constructed and used for **glucose** assay.

L17 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

2002:332347 Document No. 136:337041 Mutants of soluble **pyrroloquinoline** quinone-dependent **glucose dehydrogenase** from **Acinetobacter** and substrate specific reactivity toward sugars. Kratzsch, Peter; Schmuck, Rainer; Bunk, Daniela; Shao, Zhixin; Thym, Detlef; Knappe, Wolfgang-Reinhold (Roche Diagnostics GmbH, Germany; F. Hoffmann-La Roche Ag). PCT Int. Appl. WO 2002034919 A1 20020502, 60 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT,

BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-EP12148 20011020. PRIORITY: EP 2000-123512 20001027; EP 2000-127294 20001219.

- AB The present invention relates to improved variants of soluble **pyrroloquinoline** quinone (PQQ)-dependent **glucose dehydrogenases** (s-GDH) isolated from **Acinetobacter**. The present invention relates to mutant proteins of s-GDH with improved substrate specificity for **glucose** and/or decreased substrate specificity for **maltose**, and to different applications of these s-GDH variants, particularly for determining concns. of sugar, especially of **glucose** in a sample. Numerous rounds of mutagenic PCR and saturation mutagenesis were performed to generate mutated s-GDH. It was found and confirmed that positions 348 and 428 are of major importance and that exchange of other amino acids may further improve the specificity for **glucose** of mutated s-GDH.

L17 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

2001:910001 Document No. 136:49338 Mutagenesis of **Acinetobacter calcoaceticus glucose** dehydrogenase for increase of substrate specificity. Hayade, Hiroshi (Japan). Jpn. Kokai Tokkyo Koho JP 2001346587 A2 20011218, 11 pp. (Japanese). CODEN: JKXXAF. APPLICATION: JP 2000-172117 20000608.

- AB A. calcoaceticus **glucose** dehydrogenase (I) has a low substrate specificity and also catalyzes the dehydrogenation of lactose and **maltose**. This invention provides a process for the mutagenesis of A. calcoaceticus I in order to increase its substrate specificity. Asp-167 was substituted with other naturally occurring amino acids and some of I mutants exhibited significant improvement in substrate specificity. The mutant I can be used for the quantitation of **glucose** in food composition anal. and clin. examination

L17 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

2001:30220 Document No. 134:189977 The role of conserved His775/781 in membrane-binding PQQ **glucose** dehydrogenase of Escherichia coli and **Acinetobacter calcoaceticus**. Okuda, Junko; Yoshida, Hiromi; Kojima, Katsuhiko; Himi, Megumi; Sode, Koji (Department of Biotechnology, Faculty of Technology, Tokyo University of Agriculture and Technology, Tokyo, 184-8588, Japan). Journal of Biochemistry, Molecular Biology and Biophysics, 4(6), 415-422 (English) 2000. CODEN: JBMBF6. ISSN: 1025-8140. Publisher: Harwood Academic Publishers.

- AB A His-residue at the C-terminal region of membrane-binding PQQ **glucose** dehydrogenases is conserved as His787 in Gluconobacter oxydans, His775 in Escherichia coli and His781 in **Acinetobacter calcoaceticus**; and its functional role in enzymic activity has been proposed. Here, we constructed a series of His775/781 variants of E. coli and A. calcoaceticus PQQGDHs, and reported their enzymic characteristics. All of the variants showed different substrate specificity profiles from their wild types, and also showed decreased thermal stability. Based on these exptl. findings, together with the previous hypothesis, we concluded that the conserved His residue has a dual role: (1) this residue locates itself close to the active site and has a crucial role in determining substrate specificity, and (2) this residue locates itself at the site where it affects the conformational stability of the β -propeller fold of PQQGDH.

L17 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

2000:790643 Document No. 133:360450 Water-soluble **Acinetobacter pyrroloquinoline** quinone **glucose dehydrogenase** mutants with improved **glucose** affinity for use in **glucose** assay and sensor. Sode, Koji (Japan). PCT Int. Appl. WO

2000066744 A1 20001109, 40 pp. DESIGNATED STATES: W: CA, CN, IL, KR, US; RW: BE, DE, ES, FR, GB, IT, LU, NL. (Japanese). CODEN: PIXXD2. APPLICATION: WO 2000-JP2872 20000501. PRIORITY: JP 1999-124285 19990430; JP 2000-9137 20000118.

AB Water-soluble **Acinetobacter calcoaceticus pyrroloquinoline quinone glucose dehydrogenase** (PQQGDH-B) mutants with improved **glucose** affinity, and their genes are disclosed. Recombinant expression of those PQQGDH mutants, **glucose** assay kit and **glucose** sensor containing them are claimed. Based on the characterization of a PCR mutation of water-soluble **glucose dehydrogenase** possessing **pyrroloquinoline** quinone (PQQ), PQQGDH-B, Glu277Gly, we have constructed a series of Glu277 variants. The replacement of Glu277 to Ala, Asn, Lys, Asp, His, Gln, Val, or Gly resulted in an increase in **glucose** affinity. Km value for **glucose** of those mutants were below 10 mM, compared to 26 mM for the wild type. Mutants with substitution of other amino acids, I278F, N279H, N452T, N462D, N462K, and N462Y were also made. Those mutants also showed improved **glucose** affinity compared to the wild type. Substrate specificity of all those mutants were highest for **glucose**, compared to lactose and **maltose**. A **glucose** assay with Glu277Lys and Asn452Thr PQQGDH were developed to measure **glucose** up to 0.1 to 20 mM. A **glucose** sensor consisting of Glu277Lys and Asn452Thr PQQGDH immobilized on an electrode via carbon paste was constructed. ✓

L17 ANSWER 9 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
2000:742225 Document No. 133:307118 Water-soluble **Acinetobacter pyrroloquinoline quinone glucose dehydrogenase** mutants with improved thermal stability for use in **glucose** assay and sensor. Sode, Koji (Japan). PCT Int. Appl. WO 2000061730 A1 20001019, 43 pp. DESIGNATED STATES: W: CA, CN, IL, KR, US; RW: BE, DE, ES, FR, GB, IT, LU, NL. (Japanese). CODEN: PIXXD2. APPLICATION: WO 2000-JP2322 20000410. PRIORITY: JP 1999-101143 19990408; JP 2000-9152 20000118.

AB Water-soluble **Acinetobacter pyrroloquinoline quinone glucose dehydrogenase** (PQQGDH-B) mutants with improved thermal stability, and their genes are disclosed. Recombinant expression of those PQQGDH mutants, **glucose** assay kit and **glucose** sensor containing them are claimed. Based on the characterization of a PCR mutation of water-soluble **glucose dehydrogenase** possessing **pyrroloquinoline** quinone (PQQ), PQQGDH-B, Ser231Cys, we have constructed a series of Ser231 variants. The replacement of Ser231 to Cys, Met, Leu, Asp, Asn, His, or Lys resulted in an increase in thermal stability. Among these variants, Ser231Lys showed the highest level of thermal stability and also showed high catalytic activity. Considering that Ser231Lys showed more than an 8-fold increase in its half-life during the thermal inactivation at 55°C compared with the wild-type enzyme, and also retained catalytic activity similar to a wild-type enzyme, the application of this mutant enzyme as a **glucose** sensor constituent may develop into a stable **glucose** sensor construction. Mutants with substitution of other amino acids, I278F, Q209K, E210K, D420K, and A421D, were also made. Those mutants also showed improved thermal stability compared to the wild type. They had similar substrate specificity to the wild type enzyme, showing activity toward **glucose**, 2-deoxy-D-**glucose**, mannose, allose, 3-o-methyl-D-**glucose**, galactose, xylose, lactose, and **maltose**. A **glucose** sensor consisting of S231K PQQGDH immobilized on an electrode via carbon paste was constructed.

L17 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN
1990:435542 Document No. 113:35542 Cloning of the genes encoding the two different **glucose** dehydrogenases from **Acinetobacter calcoaceticus**. Cleton-Jansen, Anne Marie; Goosen, Nora; Vink, Kees; Van de Putte, Pieter (Lab. Mol. Genet., Univ. Leiden, Leiden, 2300 RA, Neth.).

Antonie van Leeuwenhoek, 56(1), 73-9 (English) 1990. CODEN: ALJMAO.
ISSN: 0003-6072.

- AB **Glucose dehydrogenase** (GDH) is a **pyrroloquinoline**-quinone (PQQ)- dependent bacterial enzyme which converts aldoses to their corresponding acids. *A. calcoaceticus* contains 2 different PQQ-dependent **glucose** dehydrogenases designated GDH-A which is active in vivo and GDH-B of which only in vitro activity can be shown. Genes coding for the 2 GDH enzymes were cloned. The DNA sequences of both *gdh* genes were determined. There is no obvious homol. between *gdhA* and *gdhB*. Both GDH enzymes oxidize D-**glucose** in vitro but disaccharides are specific GDH-B substrates and 2-deoxyglucose is specifically oxidized by GDH-A.

L17 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2004 ACS on STN

1987:571327 Document No. 107:171327 The in vivo and in vitro substrate specificity of quinoprotein **glucose** dehydrogenase of **Acinetobacter calcoaceticus** LMD79.41. Dokter, P.; Pronk, J. T.; Van Schie, B. J.; Van Dijken, J. P.; Duine, J. A. (Lab. Microbiol. Enzymol., Delft Univ. Technol., Delft, 2628 BC, Neth.). FEMS Microbiology Letters, 43(2), 195-200 (English) 1987. CODEN: FMLED7. ISSN: 0378-1097.

- AB Quinoprotein **glucose** dehydrogenase (GDH; EC 1.1.99.17) was partially purified from cell-free exts. of *A. calcoaceticus* LMD79.41. The enzyme oxidized monosaccharides (D-**glucose**, D-allose, 2-deoxy-D- **glucose**, D-galactose, D-mannose, D-xylose, D-ribose, and L-arabinose) as well as disaccharides (D-lactose, D-**maltose**, and D-cellobiose). Intact cells of *A. calcoaceticus* LMD79.41 also oxidized these monosaccharides, but not the disaccharides. The difference in substrate specificity can not be explained by impermeability of the outer membrane for disaccharides, since right-side-out membrane vesicles did not oxidize disaccharides either. Destruction of the cytoplasmic membrane strongly affected the catalytic properties of GDH. Not only did the affinity towards some monosaccharides change substantially, but disaccharides also became good substrates upon solubilization of the enzyme. Thus, at least in *A. calcoaceticus* LMD79.41, the oxidation of disaccharides by GDH can be considered as an in vitro artifact caused by the removal of the enzyme from its natural environment.

L18 ANSWER 1 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN

2003:115096 Document No. 139:161370 Improved substrate specificity of water-soluble **pyrroloquinoline** quinone **glucose** **dehydrogenase** by a peptide ligand. Yoshida, Hiromi; Yagi, Yukiko; Ikebukuro, Kazunori; Sode, Koji (Faculty of Technology, Department of Biotechnology, Tokyo University of Agriculture and Technology, 2-24-16 Nakamachi, Koganei, Tokyo, 184-8588, Japan). Biotechnology Letters, 25(4), 301-305 (English) 2003. CODEN: BILED3. ISSN: 0141-5492. Publisher: Kluwer Academic Publishers.

- AB A new approach in altering the substrate specificity of enzyme is proposed using **glucose** dehydrogenase, with pyrroloquinone quinone (PQQGDH) as co-factor, as the model. This approach is based on the selection of random peptide phage displayed library. Using an M13 phage-display random peptide library, we have selected peptide ligands. Among the peptide ligands, a 7-mer peptide, composed of Thr-Thr-Ala-Thr-Glu-Tyr-Ser, caused PQQGDH substrate specificity to decrease significantly toward disaccharides, such as **maltose** and lactose, while a smaller effect was observed toward **glucose**. Consequently, this peptide narrowed the substrate specificity of PQQGDH, without a significant loss of the enzyme activity.

L18 ANSWER 2 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN

2002:884721 Document No. 138:267589 Construction of Engineered Water-soluble PQQ **Glucose** Dehydrogenase with Improved Substrate Specificity. Sode, Koji; Igarashi, Satoshi; Morimoto, Akifumi; Yoshida, Hiromi (Japan). Biocatalysis and Biotransformation, 20(6), 405-412 (English) 2002. CODEN: BOBOEQ. ISSN: 1024-2422. Publisher: Taylor & Francis Ltd..

AB This was the first study that achieved a narrowing of the substrate specificity of water soluble **glucose dehydrogenase** harboring **pyrroloquinoline** quinone as their prosthetic group, PQQGDH-B. We conducted the introduction of amino acid substitutions into the loop 6BC region of the enzyme, which made up the active site cleft without directly interacting with the substrate, and constructed a series of site directed mutants. Among these mutants, Asn452Thr showed the least narrowed substrate specificity while retaining a similar catalytic efficiency, thermal stability and EDTA tolerance as the wild-type enzyme. The relative activities of mutant enzyme with lactose were lower than that of the wild-type enzyme. The altered substrate specificity profile of the mutant enzyme was found to be mainly due to increase in Km value for substrate than **glucose**. The predicted 3D structures of Asn452Thr and the wild-type enzyme indicated that the most significant impact of the amino acid substitution was observed in the interaction between the 6BC loop region with lactose.

L18 ANSWER 3 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN

2002:89878 Document No. 136:156403 Methods for identifying therapeutic targets for treating infectious disease. Shepard, Michael H.; Lackey, David B.; Cathers, Brian E.; Sergeeva, Maria V. (Newbiotics, Inc., USA). PCT Int. Appl. WO 2002007780 A2 20020131, 503 pp. DESIGNATED STATES: W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM; RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, CY, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG, TR. (English). CODEN: PIXXD2. APPLICATION: WO 2001-US23095 20010720. PRIORITY: US 2000-PV219598 20000720; US 2000-PV244953 20001101; US 2001-PV276728 20010316.

AB This invention provides methods and systems to identify enzymes that act as enzyme-catalyzed therapeutic activators and the enzymes identified by these methods. Also provided by this invention are compds. activated by the enzymes as well as compns. containing these compds.

L18 ANSWER 4 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN

1998:577611 Document No. 129:299584 On the Mechanism and Specificity of Soluble, Quinoprotein **Glucose** Dehydrogenase in the Oxidation of Aldose Sugars. Olsthoorn, Arjen J. J.; Duine, Johannis A. (Department of Microbiology and Enzymology, Delft University of Technology, Delft, 2628 BC, Neth.). Biochemistry, 37(39), 13854-13861 (English) 1998. CODEN: BICHAW. ISSN: 0006-2960. Publisher: American Chemical Society.

AB Kinetic and optical studies were performed on the reductive half-reaction of soluble, quinoprotein **glucose** dehydrogenase (sGDH), i.e., on the conversion of sGDHox plus aldose sugar into sGDHred plus corresponding aldonolactone. It appears that the nature and stereochem. configuration of the substituents at certain positions in the aldose mol. determine the substrate specificity pattern: absolute specificity exists with respect to the C1-position (only sugars being oxidized which have the same configuration of the H/OH substituents at this site as the β -anomer of **glucose**, not those with the opposite one) and with respect to the overall conformation of the sugar mol. (sugars with a 4C1 chair conformation are substrates, those with a 1C4 one are

not); the nature and configuration of the substituents at the 3-position are hardly relevant for activity, and an equatorial pyranose group at the 4-position exhibits only aspecific hindering of the binding of the aldose moiety of a disaccharide. The pH optimum determined for **glucose** oxidation appeared to be 7.0, implying that reoxidn. of sGDHred is rate-limiting with those electron acceptors displaying a different value under steady-state conditions. The kinetic mechanism of sGDH consists of (a) step(s) in which a fluorescing intermediate is formed, and a subsequent, irreversible step, determining the overall rate of the reductive half-reaction. The consequences of this for the likeliness of chemical mechanisms where **glucose** is oxidized by covalent catalysis in which a C5-adduct of **glucose** and PQQ (pyrroloquinoline quinone) are involved, or by hydride transfer from **glucose** to PQQ, followed by tautomerization of C5-reduced PQQ to PQQH2, are discussed. The neg. cooperative behavior of sGDH seems to be due to substrate-occupation-dependent subunit interaction in the dimeric enzyme mol., leading to a large increase of the turnover rate under saturating conditions.

L18 ANSWER 5 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN

1998:557951 Document No. 129:256919 Mutant isolation of the Escherichia coli quinoprotein **glucose** dehydrogenase and analysis of crucial residues Asp-730 and His-775 for its function. Yamada, Mamoru; Inbe, Hisayo; Tanaka, Makoto; Sumi, Kenichi; Matsushita, Kazunobu; Adachi, Osao (Department of Biological Chemistry, Faculty of Agriculture, Yamaguchi University, Yamaguchi, 753-8515, Japan). Journal of Biological Chemistry, 273(34), 22021-22027 (English) 1998. CODEN: JBCHA3. ISSN: 0021-9258. Publisher: American Society for Biochemistry and Molecular Biology.

AB Several mutants of quinoprotein **glucose** dehydrogenase (GDH) in Escherichia coli were obtained and characterized. Of these, significant mutants were further characterized by kinetic anal. after purification or by site-directed mutagenesis to introduce different amino acid substitutions. H775R and H775A showed a pronounced reduction of affinity for a prosthetic group, pyrroloquinoline quinone (PQQ), suggesting that His-775 may directly interact with PQQ. D730N and D730A showed low **glucose** oxidase activity without influence on the affinity for PQQ, Mg2+, or substrate, but D730R showed reduced affinity for PQQ. The spectrum of tryptophan fluorescence revealed that the local structure surrounding PQQ was not changed by D730N mutation. Based on these data, we assume that Asp-730 may occur close to PQQ and function as a proton (and also electron) donor to PQQ or acceptor from PQQH2. Substitutions of Gly-689, that are located at the end of a unique segment of GDH among homologous quinoprotein dehydrogenases, directed reduction of the affinity for PQQ or GDH activity. Therefore, the unique segment and Asp-730 may play a specific role for GDH, which might be related to the intramol. electron transfer from PQQ to ubiquinone. ✓

L18 ANSWER 6 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN

1994:503582 Document No. 121:103582 Method for the simple enzymic detection or determination of inorganic phosphate. Warsinke, Axel; Gruendig, Bernd (Institut fuer Chemo- und Biosensorik, Germany). Ger. DE 4227569 C1 19940609, 8 pp. (German). CODEN: GWXXAW. APPLICATION: DE 1992-4227569 19920820.

AB The title method is especially suitable for the production of biosensors or test strips whereby the method can be applied either directly for the determination of phosphate or indirectly as an indicator of phosphate-releasing reactions. The invention uses an enzymic amplification system that is composed of 3 enzymes: a disaccharide phosphorylase, a **glucose** 1-phosphate-hydrolyzing enzyme, and a **glucose** oxidoreductase. By combination of the disaccharide phosphorylase and the **glucose** 1-phosphate-hydrolyzing enzyme, a large number of **glucose** mols. are generated for each mol. of inorg. phosphate,

and the **glucose** is subsequently determined via the **glucose** oxidoreductase reaction. The invention is especially useful for environmental anal., tech. microbiol., and clin. anal. Thus, for phosphate determination with a biosensor, an enzyme layer was prepared by coimmobilizing **maltose** phosphorylase 10, acid phosphatase 10, and **glucose** oxidase 5 units per 1 cm² gelatin. The enzyme membrane was fixed between 2 dialysis membranes on an amperometric electrode that detects H₂O₂. The biosensor was dipped into a stirred measuring cell, and 2 mL of 50 mM **maltose** solution in 50 mM citrate buffer (pH 5.0) was pipetted into the cell. Then different concns. of NaH₂PO₄ were pipetted into the solution and a calibration curve was prepared

L18 ANSWER 7 OF 7 CAPLUS COPYRIGHT 2004 ACS on STN

1992:629010 Document No. 117:229010 A single amino acid substitution changes the substrate specificity of quinoprotein **glucose** dehydrogenase in *Gluconobacter oxydans*. Cleton-Jansen, Anne Marie; Dekker, Sylvia; Van de Putte, Pieter; Goosen, Nora (Lab. Mol. Genet., Univ. Leiden, Leiden, 2300 RA, Neth.). Molecular and General Genetics, 229(2), 206-12 (English) 1991. CODEN: MGGEAE. ISSN: 0026-8925.

AB *G. oxydans* contains **pyrroloquinoline** quinone-dependent **glucose dehydrogenase** (GDH). Two isogenic *G. oxydans* strains, P1 and P2, which differ in their substrate specificity with respect to oxidation of sugars have been analyzed. P1 can oxidize only D- **glucose**, whereas P2 is also capable of the oxidation of the disaccharide **maltose**. To investigate the nature of this **maltose**-oxidizing property the authors cloned the gene encoding GDH from P2. Expression of P2 gdh in P1 enables the latter strain to oxidize **maltose**, indicating that a mutation in the P2 gdh gene is responsible for the change in substrate specificity. This mutation could be ascribed to a 1 bp substitution resulting in the replacement of His 787 by Asn.

=> S L10 AND L13

L19 79 L10 AND L13

=> S L19 AND 348; S L19 AND 428

7331 348

L20 1 L19 AND 348

5274 428

L21 3 L19 AND 428

=> S L20, L21

L22 3 (L20 OR L21)

=> S L22 NOT (L17, L18)

L23 1 L22 NOT ((L17 OR L18))

=> D CBIB ABS

L23 ANSWER 1 OF 1 CAPLUS COPYRIGHT 2004 ACS on STN

1988:506763 Document No. 109:106763 Cytochrome b-562 from **Acinetobacter calcoaceticus** L.M.D. 79.41. Its characteristics and role as electron acceptor for quinoprotein glucose dehydrogenase. Dokter, Paul; Van Wielink, John E.; Van Kleef, Mario A. G.; Duine, Jhannis A. (Lab. Microbiol. Enzymol., Delft Univ. Technol., Delft, 2628 BC, Neth.). Biochemical Journal, 254(1), 131-8 (English) 1988. CODEN: BIJOAK. ISSN: 0306-3275.

AB A soluble cytochrome b was purified from *A. calcoaceticus* L.M.D. 79.41. On the basis of the α -band maximum of a reduced preparation, measured at 25°, it is designated as cytochrome b 562. This cytochrome is a basic monomeric

protein (pI 10.2; Mr 18,000), containing 1 protoheme group per mol. The reduced form, at 25°, showed absorption bands at 428, 532, and 562 nm. At 77 K the α -band shifted to 560 nm (with a shoulder at 558 nm). The reduced cytochrome did not react with CO. Cytochrome b 562 is probably loosely attached to the outside of the cytoplasmic membrane, since substantial amts. of it, equimolar to quinoprotein glucose dehydrogenase (GDH), were in the culture medium when cells were grown in the presence of low concns. of Triton X-100. The midpoint potential at pH 7.0 was +170 mV, a value that was lowered to +145 mV by the presence of GDH. Since the GDH was shown to have a midpoint potential of +50 mV, cytochrome b 562 could function as the natural primary electron acceptor. Arguments to substantiate this view and to propose a role of ubiquinone-9 as electron acceptor for cytochrome b 562 are presented.

=> E KRATZCH P/AU

=> S E4

L24 1 "KRATZCH PETER"/AU

=> E SCHMUCK R/AU

=> S E4,E5

1 "SCHMUCK R F"/AU

20 "SCHMUCK RAINER"/AU

L25 21 ("SCHMUCK R F"/AU OR "SCHMUCK RAINER"/AU)

=> E BUNK D/AU

=> S E5

L26 4 "BUNK DANIELA"/AU

=> E SHAO Z/AU

=> S E3,E98

58 "SHAO Z"/AU

15 "SHAO ZHIXIN"/AU

L27 73 ("SHAO Z"/AU OR "SHAO ZHIXIN"/AU)

=> E THYM D/AU

=> S E3-E5

1 "THYM D"/AU

8 "THYM DETLEF"/AU

1 "THYM DETLFE"/AU

L28 10 ("THYM D"/AU OR "THYM DETLEF"/AU OR "THYM DETLFE"/AU)

=> E KNAPPE W/AU

=> S E3,E4,E8-E10

50 "KNAPPE W"/AU

10 "KNAPPE W R"/AU

3 "KNAPPE WOLFGANG"/AU

17 "KNAPPE WOLFGANG R"/AU

17 "KNAPPE WOLFGANG REINHOLD"/AU

L29 97 ("KNAPPE W"/AU OR "KNAPPE W R"/AU OR "KNAPPE WOLFGANG"/AU OR "KNAPPE WOLFGANG R"/AU OR "KNAPPE WOLFGANG REINHOLD"/AU)

=> S L24,L25,L26,L27,L28,L29

L30 191 (L24 OR L25 OR L26 OR L27 OR L28 OR L29)

=> S L30 AND L10

L31 5 L30 AND L10

=> S L31 NOT (L17,L18,L23)

L32 3 L31 NOT ((L17 OR L18 OR L23))

=> D 1-3 CBIB ABS

L32 ANSWER 1 OF 3 CAPLUS COPYRIGHT 2004 ACS on STN

2004:18838 Document No. 140:92687 Forms of soluble **pyrroloquinoline**

quinone-dependent **glucose dehydrogenase**. Kratzsch, Peter; Schmuck, Rainer; Beck, Daniela; Shao, Zhixin; Thym, Detlef; Knappe, Wolfgang-Reinhold (Germany). U.S.

Pat. Appl. Publ. US 2004005683 A1 20040108, 31 pp., Cont.-in-part of U.S. Ser. No. 82,627. (English). CODEN: USXXCO. APPLICATION: US 2002-319147 20021213. PRIORITY: EP 2000-123512 20001027; US 2000-710197 20001109; EP 2000-127294 20001219; US 2001-82627 20011029.

AB The present invention relates to improved variants of soluble **pyrroloquinoline** quinone-dependent **glucose dehydrogenases** (s-GDH), to genes encoding mutated s-GDH, to mutant proteins of s-GDH with improved substrate specificity for glucose, and to different applications of these s-GDH variants, particularly for determining concns. of sugar, especially of glucose in a sample.

L32 ANSWER 2 OF 3 CAPLUS COPYRIGHT 2004 ACS on STN

2000:277756 Document No. 132:290743 Preparation of diagnostic test strips that contain a reagent layer directly coated with a wetting agent.

Knappe, Wolfgang; Mosoiu, Dan (Roche Diagnostics G.m.b.H., Germany). Eur. Pat. Appl. EP 995994 A2 20000426, 18 pp. DESIGNATED STATES: R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO. (German). CODEN: EPXXDW. APPLICATION: EP 1999-120663 19991019. PRIORITY: DE 1998-19849000 19981023.

AB The invention concerns diagnostic test strips that are coated directly on the top of the reagent layer with wetting agents containing one of the following compds.: $R_1\text{-CO-N}(R_2)\text{-CH}_2\text{-COOMe}$; $R_1\text{-CO-N}(R_2)\text{-CH}_2\text{-CH}_2\text{-SO}_3\text{Me}$; $\text{HO}_2\text{C-CH}_2\text{-CH}_2\text{-CH}(\text{NH-COR}_1)\text{-CO}_2\text{Me}$, where R_1 = C₉-C₂₅ linear or branched aliphatic group, preferably C₁₁-C₁₉; R_2 = C₁-C₈ alkyl; Me = H or metal, e.g. potassium, sodium. The wetting agent containing composition is directly placed on the top of the reagent layer without a support, e.g. textile. Thus a solution of Geropon T77 was applied on the top of a layer that was impregnated with reagents for glucose assay.

L32 ANSWER 3 OF 3 CAPLUS COPYRIGHT 2004 ACS on STN

1998:79705 Document No. 128:151448 Diagnostic sample holder and multilayer test kit withholding erythrocytes on the sample application side and being transparent on the detection side. **Thym, Detlef**; **Knappe, Wolfgang-Reinhold**; Pacht, Rudolf; Merdes, Hartmut; Lorenz, Robert (Boehringer Mannheim G.m.b.H., Germany). Ger. Offen. DE 19629656 A1 19980129, 18 pp. (German). CODEN: GWXXBX. APPLICATION: DE 1996-19629656 19960723.

AB The invention concerns a diagnostic sample holder and test kit for the colorimetric measurement of whole blood, that consists of a base, a transparent support foil, two layers that contain the diagnostic reagents and a hydrophilic mesh cover. The layer at the sample application side consists of a film that is non permeable for the erythrocytes, thus interferences are avoided during the diagnosis reaction. At least 25 % of this layer's dry weight is a pigment with a refractive index of min. 2.5, e.g. TiO₂; the layer on the detection side has a much lower refractive index. The two layers are maximum 0.2 mm when dry, the layer with the high refractive index being 2-5 times thicker than the other. The layers are prepared from the same or different polymers, and these polymers can be formed from a dispersion or an emulsion; the crosslinking agent must not cause hemolysis, thus N-octanoyl-methyl-glucamide is applied. The base has usually a hole below the test field; thus the color change is detected by reflectance, and data are evaluated using calibrations. Polymers used for the detection layers can be homopolymers or copolymers of polyvinylacetate, polyacrylic ester, polymethacrylic acid, polyvinylamide, polyamide, and polystyrene, and the transparent support is

plycarbonate. The diagnostic layers contain filling materials, e.g. silicates, Na-aluminosilicate, and swelling components, e.g. methylvinylether-maleic acid anhydride copolymer, methylvinylether-maleic acid copolymer, and xanthan gum. Thus glucose is measured from whole blood with a kit containing in one of the layers recombinant glucose dehydrogenase from *Acinetobacter calcoaceticus* and pyrroloquinoline-quinone.

	L #	Hits	Search Text	DBs
1	L1	881	GLUCOSE ADJ DEHYDROGENASE	USPAT ; US-PG PUB
2	L2	279	PYRROLOQUINOLINE	USPAT ; US-PG PUB
3	L4	2316	ACINETOBACTER	USPAT ; US-PG PUB
4	L5	5	L3 AND L4	USPAT ; US-PG PUB
5	L6	50815	MUTANT	USPAT ; US-PG PUB
6	L7	1	L3 NEAR6 L6	USPAT ; US-PG PUB
7	L3	46	L1 NEAR5 L2	USPAT ; US-PG PUB